IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Hisakazu KATSUKI et al
Application No.: 10/588,609
Filed: August 7, 2006
For: ED-71 PREPARATION

Confirmation No. 8366
Art Unit: 1616

Examiner: QAZI, SABIHA NAIM

DECLARATION

Honorable Commissioner of Patent and Trademarks
P.O.Box 1450
Alexandria, Virginia 22313-1450

Sir:

I, Hitoshi SAITO, a Japanese citizen, residing at 33-3 Fuji Village, Mishima-city, Shizuoka-prefecture, Japan, hereby solemnly and sincerely declare and state that:

I was awarded M.Sc. in 1989 from the Faculty of Science and Technology, Science University of Tokyo, Chiba-prefecture, Japan;

I have been employed by Chugai Pharmaceutical Co. Ltd., the assignee of the present application, since 1997, and worked at Fuji-Gotemba Research Laboratories in Gotemba, Shizuoka, Japan from 1997, as a researcher of bone biology field during the entire period.

I declare further that I engaged as a researcher in research into vitamin D3 preparations.

I declare further that I have read the Official Action in the above-identified application, and have read, and am familiar with each of the references cited in the Official Action by the Examiner.

Purpose of this declaration

The purpose of this declaration is to show experimental data to establish an advantageous effect of (5E,7E)-(1R,2R,3R)-2-(3-hydroxypropoxy)-9,10-secocholesta-5,7,10(19)-triene-1,3,25-triol (trans form of ED-71).

I declare that the following tests were conducted at my direction or under my supervision, and that the test results are true and correct to the best of my knowledge.

Materials and Test Method

1. Test Samples

Trans form of ED-71 (lot No.: YM99G045-4-1)

Site of manufacture: Chugai Pharmaceutical Co. Ltd, Synthetic Technology Research Laboratory

Supplier: Chugai Pharmaceutical Co. Ltd, Synthetic Technology Research Laboratory

Supplied form: ethanol solution (1mg/mL)

Storage conditions: in a light-shielding vial, in a freezer at a preset temperature of –20℃ or less

Control 1: 1α, 25-$(OH)_2$-D_3 (lot No.: WVC99AJ87)

Manufacturer: SOLVAY PHARMACEUTICALS

Supplier: Chugai Pharmaceutical Co. Ltd, Chemistry Research Laboratory

Supplied form: ethanol solution (1.02mg/mL)

Storage conditions: in a light-shielding vial, in a freezer at a preset temperature of –20℃ or less

Comments: This substance was selected to show an effect of a representative active vitamin D as a positive control, for comparison with trans form of ED-71.

Control 2: ED-71 (lot No.: 8G03ED)

Site of manufacture: Chugai Pharmaceutical Co. Ltd, Synthetic Technology Research Laboratory

Supplier: Chugai Pharmaceutical Co. Ltd, Synthetic Technology Research Laboratory

Supplied form: powder (25mg)

Storage conditions: in a light-shielding vial, in a freezer at a preset temperature of –20℃ or less

Used form: Before testing ED-71, a ED-71 solution in ethanol (0.93mg/mL)

was prepared, and stored in a light-shielding vial filled with argon gas, in a freezer at a preset temperature of –20℃ or less. Ethanol was selected since it is commonly used as a solvent for vitamin D derivatives.

Comments: ED-71 substance is a parent compound of trans form of ED-71.

2. Cells used

Cells: HL-60 cells

Subculture method: subculture was conducted in RPMI-1640 medium supplemented with 10% of fetal calf serum, at 37℃, in an atmosphere of 5% CO_2 in air.

Storage location: -130℃ freezer

Origin (animal species): human acute myelogenous leukemia cell strain (supplied by Chugai Pharmaceutical Co. Ltd, Pharmaceutical Technology Research Laboratory

3. Experimental Method

HL-60 cells were subcultured in RPMI-1640 medium supplemented with 10% of heat-inactivated fetal calf serum and 20μg/ml gentamycin, at 37℃, in a humidified atmosphere of 5% CO_2 in air.

An ability of induction of differentiation was estimated by the ability of HL-60 cells to generate a superoxide anion.

Each of the solutions of control samples (1α, 25-$(OH)_2$-D_3 and ED-71) and trans form of ED-71 (about 10^{-3} mg/mL) was diluted by use of 10 volumes of RPMI-1640 medium seven times sequentially, to produce sample solutions with a concentration of $1x10^{-10}$-$1x10^{-4}$mg/mL. HL-60 cells were seeded at $1x10^5$cells/mL in a growth media and cultured for 4 days in the presence of various concentrations of the sample solutions, to induce differentiation. Then, the cells were washed free of the compounds, and suspended in a 1.5mL reaction mixture containing 80μM ferricytochrome c (Sigma Chemical Co., St. Louis, MO; Sigma code: C-2506), and 500 ng/mL phorbol myristate acetate (Sigma; Sigma code: P-8139) in 0.1% gelatin Hank's balanced salt solution without phenol red. The mixture was incubated at 37℃ for 60 min, and centrifuged for 10 min. at 400xg at 4℃. The reduction of ferricytochrome c was measured by use of the absorption increase at 550 to 540 nm (molar absorption coefficient, $19.1x10^3$/cm) with a Hitachi U-3200 double-beam spectrometer. The results are shown in Figure 1 and Table 1 below.

Results

Fig 1 Differentiation-Inducing activity on HL-60 cells

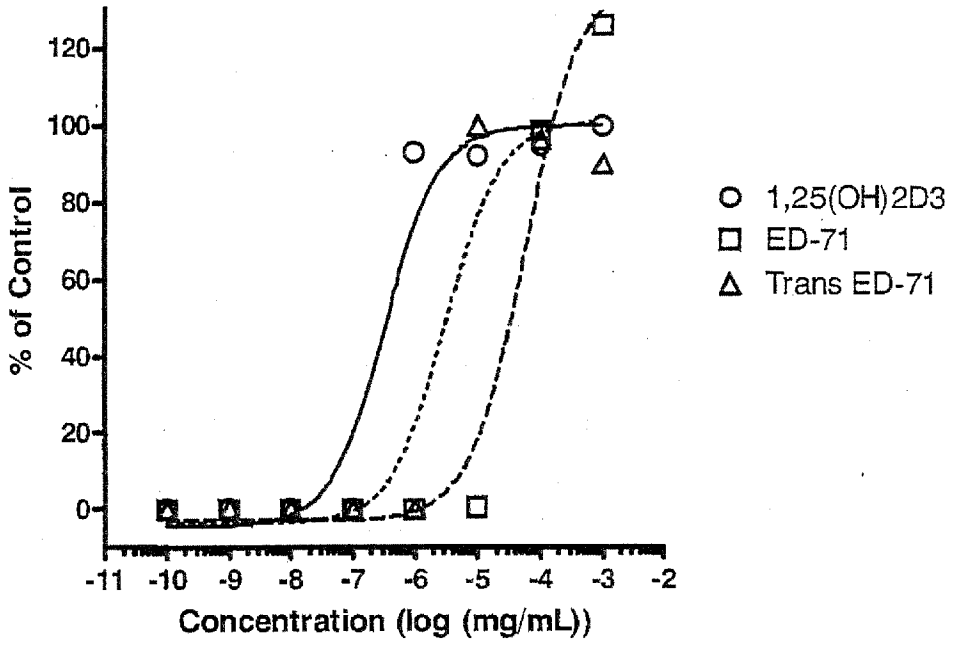


Table 1 Comparison of differentiation-inducing activity on HL-60 cells

Vitamin D derivatives	EC_{50}	Relative differentiation-inducing activity calculated based on 1α, 25-$(OH)_2$-D_3
1α, 25-$(OH)_2$-D_3	4.91×10^{-7}	1
ED-71	5.77×10^{-5}	0.0085
Trans form of ED-71	2.84×10^{-6}	0.1731

As shown in Table 1, trans form of ED-71 showed a relative differentiation-inducing activity on HL-60 cells of 0.1731 (this value was calculated from EC_{50} of the trans form, on the basis of EC_{50} of 1α, 25-$(OH)_2$-D_3, with regarding EC_{50} of 1α, 25-$(OH)_2$-D_3 as "1"). This value is almost 20 times higher than that of the parent compound, ED-71.

Conclusion

Trans form of ED-71 of the present invention shows a differentiation-inducing activity of almost 20 times higher than that of the parent compound, ED-71. I consider that this fact

supports an advantageous effect of the present invention beyond expectations of those skilled in the art. Therefore, I trust that the present invention of trans form of ED-71 is unobvious over the citations.

I declare further that all statements made therein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the instant patent application or any patent issuing thereon..

Dated this 29th day of January, 2008

Hitoshi SAITO

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

		Atty. Docket: KATSUKI2
In re Application of:	)	Conf. No.: 8366
Hisakazu KATSUKI et al.	)	Art Unit: 1616
Appln. No.: 10/588,609	)	Examiner: S. N. Qazi
Filed: August 7, 2006	)	Washington, D.C.
For: ED-71 PREPARATION	)	

DECLARATION UNDER 37 CFR 1.132

Honorable Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22314

Sir:

I, Hisakazu Katsuki, a Japanese citizen, hereby declare as follows:

1. I received a master's degree in Pharmaceutical Science (Clinical pharmacy course) in March 1995, at Kumamoto University, Kumamoto-shi, Japan.

2. I have been employed by Chugai Pharmaceutical Co. Ltd., the Assignee of this application, since 1995, and I have worked as a researcher for the Ukima Research Laboratories of the Assignee, at Kita-ku, Tokyo, Japan.

3. I have read the Official Action issued against the subject patent application mailed on March 20, 2009 and have noted the Examiner's allegation that Claims 12 and 13 are rejected under 35 U.S.C. 103(a) as being unpatentable

over CHEN et al. (WO 03/047595), MIYAMOTO et al. (US Patent 4,666,634) and Chem. Pharm. Bull. (all 892 references).

4. I, one of inventors of the subject application, conducted the following experiment, in order to evidence that the method of Claims 12 and 13 achieves results greater than those which would have been expected from the combination of the prior arts.

5. The results are true and correct to the best of my knowledge.

Method:

To compare anticxidants, BHA, catechin, tocopherol, ferulic acid, BHT, citric acid, and thiolactic acid, with one another with respect to effectiveness in suppressing generation of (5E,7E)-(1R,2R,3R)-2-(3-hydroxypropoxy)-9,10-secocholesta-5,7,10(19)-triene-1,3,25-triol (hereinafter referred to as "trans form of ED-71") in an oily preparation containing (5Z,7E)-(1R,2R,3R)-2-(3-hydroxypropoxy)-9,10-secocholesta-5,7,10(19)-triene-1,3,25-triol (hereinafter referred to as "ED-71"), soft capsules filled with a solution of ED-71 and one of the antioxidants in a medium-chain triglyceride, caprylic/capric triglyceride (commercially available as "MCT(ODO-C)"), were prepared and stored under conditions for accelerated degradation of ED-71. After the storage, the capsules were each analyzed for the amount of trans form of ED-71 formed during the storage.

1) Preparation of soft capsules

ED-71 and one of the antioxidants were dissolved in caprylic/capric triglyceride to prepare a solution containing 1 μg (2 nmol) of ED-71 and 11.9 nmol of the antioxidant per 100 mg of the solution. Then, 100 mg of the solution was injected into an empty soft capsule, the shell of which is composed of 54.71 mg of gelatin, 8.34 mg of D-sorbitol and 1.95 mg of caramel, by means of a syringe with a needle. The capsule was sealed with gelatin.

These procedures were repeated for each of the antioxidants to provide sealed soft capsules containing various types of antioxidant.

2) Storage

The sealed soft capsules were placed into a bottle which was then sealed. The bottle was stored at 40°C for two months.

3) Determination of trans form of ED-71

After completion of storage, the sealed soft capsules were removed from the bottle, and the solutions were extracted from the capsules, and 50-μL aliquots thereof were then subjected to HPLC analysis to determine the amount of trans form of ED-71 formed during the storage.

Column: YMC-Pack ODS AM-303 (250 × 4.6 mm, 5 μm)

Mobile phase: acetonitrile/water =1:1

Flow rate: 1.2 mL/min

Peak detection: 265 nm

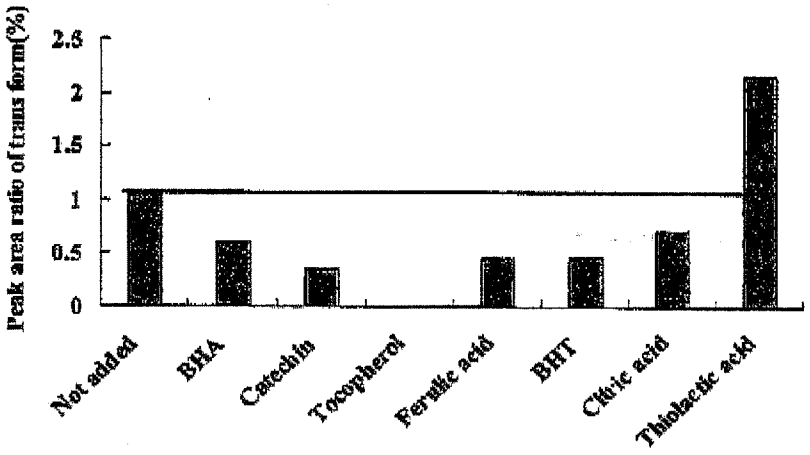
Column temperature: 30°C

Running time: 35 min

A peak area ratio of trans form of ED-71 relative to the total sum of detected peak areas was calculated and used as an index of the amount of trans form of ED-71.

Results:

The figure shown below is a graphical presentation of the results obtained. The lower the level of the bar in the figure, the greater the effectiveness of the antioxidant designated immediately below the bar in suppressing the formation of trans form of ED-71.



As can be seen form the figure, a dramatic effect on the suppression of the formation of trans form of ED-71 is observed when tocopherol is added. Tocopherol is far more effective than the other antioxidants tested, i.e., BHA, catechin, ferulic acid, BHT, citric acid and thiolactic acid.

The results indicate that the effect of the inventive method currently claimed in Claims 12 and 13 where antioxidants are limited to dl-α-tocopherol is greater than that which would have been expected from a combination of the prior arts, and that the effect is of a significant, practical advantage. Such an effect would not have been expected from the disclosures of the prior arts.

Therefore, the currently claimed method is unobvious over the prior arts.

I hereby further declare that all statements made herein are to my own knowledge and belief true, and that all statements made on information and belief are believed to be true, and that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

August 20, 2009 Hisakazu Katsuki

Hisakazu Katsuki